



Depolymerized Chitosan Enhances the Lysis of *Staphylococcus aureus* Cells by Lysostaphin

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1 Introduction

The improper use of antibiotics in the treatment of bacterial infections has resulted in the appearance of spreading resistant strains [1]. Among them, *Staphylococcus aureus* has been recognized as an important pathogen both in community-acquired and healthcare-associated infections. *S. aureus* has successfully become resistant to practically all antibiotics, and this is a serious clinical problem in the world today [2]. Therefore, the search for new pharmaceuticals alternative to antibiotics and design of new treatment approaches of staphylococcal infections are important tasks.

Lysostaphin is a zinc metalloenzyme which has a specific lytic action against *S. aureus*. Glycylglycine endopeptidase specifically cleaves the glycine–glycine bonds, unique to the interpeptide cross-bridge of the *S. aureus* cell wall. Due to its unique specificity, lysostaphin could have high potential in the treatment of antibiotic-resistant staphylococcal infections [3].

It is known that lysostaphin shows synergistic effects in its antistaphylococcal activity when used in combination with conventional antibiotics [4–6], antimicrobial peptides [7], and proteins [8]. This makes it possible to reduce the amount of the used enzyme and retain high antistaphylococcal activity. Since antibacterial proteins lead to acquired resistance in *Staphylococcus* strains to a much lesser degree, they may have

significant advantages over traditional antibiotics [9]. It was found previously that high-molecular weight chitosan (HMWC) can enhance lysostaphin-mediated cell lysis of *S. aureus* cells [10]. However, HMWC has several disadvantage such as poor solubility and high viscosity. So, we studied the effect of low molecular weight chitosan (LMWC) on the lysis of living staphylococcal cells by lysostaphin.

2 Experimental

2.1 Materials and Methods

2.1.1 Preparation of Low Molecular Weight Chitosan

We used crab HMWC with an average molecular weight (M_v) of 600 kD and a deacetylation degree of 85% (ZAO Bioprogress, Russia). High-molecular weight chitosan was hydrolyzed in hydrochloric acid at 70 °C, and LMWC hydrochloride was precipitated with ethanol and dried in vacuum over sodium hydroxide as described [11]. Chitosan solutions were filtrated through a 0.22 μm poresize syringe filter (Millipore, Swinnox) and stored at 4 °C until usage.

2.1.2 Chitosan Characteristics

The medium-viscosity molecular weight of chitosan was calculated by Mark-Coon-Houwink equation for chitosans with different degree of deacetylation as described previously [12]; the deacetylation degree was evaluated according to [13].

2.1.3 Microorganism

S. aureus ATCC 35591 was stored in a semiliquid meat peptone agar (MPA) at 4 °C. For the growth of bacteria, 5% suspension of bacterial culture was added in a 100 mL flask containing 20 mL of meat peptone broth (MPB) and incubated with shaking at 150 rev/min for 18 h at 37 °C.

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